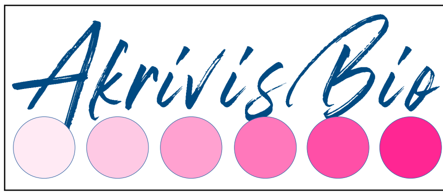


MA-0138

α-Amylase Activity Assay

(10 wells, Colorimetric, OD 405 nm, Store at 20°C)

Introduction:

Amylases hydrolyze starch and glycogen into maltose and oligosaccharides. α-Amylase, the major form of amylase found in humans is a calcium metallo-enzyme. Enzymes from other species have different metal ion requirements. A variety of disease states cause changes in amylase levels, including salivary gland trauma or inflammation, mumps, pancreatitis and renal failure. AkrivisBio's α-Amylase Activity Assay is a simple, sensitive means of measuring amylase activity. In the assay, amylase cleaves ethylidene-pNP-G7 to smaller oligosaccharides which are subsequently hydrolyzed by α-glucosidase, releasing para-nitrophenol a chromophore measured at 405 nm. Amylase activity at levels below 1 mU.

Assay Principle:

1 – Amylase hydrolyzes the artificial substrate ethylidene-pNP-G7 to small oligosaccharides
2 – glucoamylase (α-glucosidase) hydrolyzes the oligosaccharides releasing nitrophenol with absorbance at 405 nm.

Assay Components:

Assay Buffer	55 ml	NM	MA-0138-A
ethylidene-pNP-G7	5 ml	NM	MA-0138-B
Amylase Positive Control	lyoph	Red	MA-0138-C
Nitrophenol Standard	150 µl	Yellow	MA-0138-D

Storage and Handling:

Store unopened assay at -20°C. Thaw frozen components at room temperature before using. Centrifuge all small vials for a few seconds prior to using. Keep samples and Amylase positive control on ice while in use.
Assay Buffer: Ready to use as supplied. Bring to room temperature to sue. Store at 4°C
Ethylidene-pNP-G7: Ready to use as supplied. Store at 4°C
Amylase Positive Control: Reconstitute with 50 µl of Assay Buffer. If the assay is to be used repeatedly over a period of time, aliquot into convenient portions and store at -20°C.
Nitrophenol standard: Ready to use as supplied. Store at 4°C

Assay Protocol:

1. Standard Curve: Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of the nitrophenol standard to a series of wells in a 96 well plate. Adjust all well volumes to 50 µl with DI water, giving 0 – 4 – 8 – 12 – 16 – 20 nmoles nitrophenol.

2. Positive Control: Transfer 5 µl to a well of a 96 well plate. Adjust well volume to 50 µl with Assay Buffer.

3. Sample Preparation: Transfer 1 – 50 µl of serum or urine directly to wells of a 96 well plate. Homogenize tissue (100 mg) or cells (4 x 10^6) in 0.5 ml Assay Buffer and centrifuge at 16,000 X g for 5 min. Transfer the clear supernatant to a fresh tube. Use 5-50 µl per well.

4. Initiate Reaction: Each well requires 100 µl Reaction Mix. Prepare sufficient volume for the number of sample, standard and positive control wells to be run

Reaction Mix:

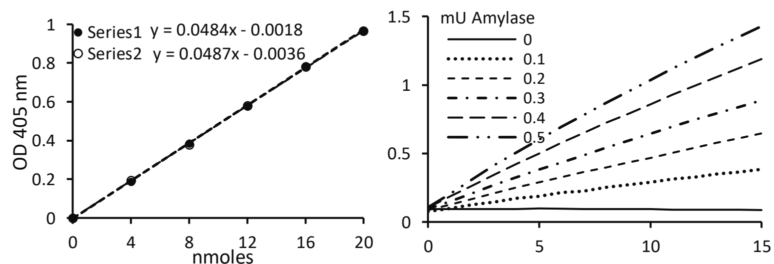
Assay Buffer	50 µl
Substrate Mix	50 µl

Transfer 100 µl of reaction mix to each well.

5. Measurement: Start monitoring the reactions immediately at 25°C on a plate reader at 405 nm in kinetic mode. Continue until a significant change in absorbance has been observed (> 0.2 OD). The OD of the standards will not change over time.

6. Typical Results:

nmole Standard	Standard Raw Values (OD)		Background Corrected Values (OD)	
0	0.0721	0.0706	0	0
4	0.2607	0.2652	0.1886	0.1946
8	0.4566	0.4481	0.3845	0.3775
12	0.6481	0.6495	0.5760	0.5789
16	0.8585	0.8506	0.7864	0.7800
20	1.0401	1.0356	0.9680	0.9650



7. Calculations: Subtract the 0 nitrophenol standard from all the other standard values. Plot the Nitrophenol Standard Curve. Determine the slope of the Standard curve. This will define the system response to nitrophenol in the well in terms of OD/nmole. Determine the time period where the enzyme rate is changing linearly with time. There may be an initial lag and a later slowing of reaction due to decreased substrate availability and these should be excluded from the rate determination. Determine the slope of the linear phase which will be in OD/minute. Divide the enzyme rate slope by the slope of the standard curve to give a rate in nmoles/minute (= mU) in the well. To convert back to mU/sample:

A. Divide the mU by the volume added to the well in µl = mU/µl of sample
B. Multiply the mU/µl of sample X total volume in µl of the clear supernatant after centrifugation in step 3 above = total mU per sample
C. Divide the total mU per sample by the mg tissue (or # of cells or µl of fluid sample) to get mU/mg of tissue (or per # of cells, etc.)

FOR RESEARCH USE ONLY! Not to be used on humans.